



Fig. 1. Cross-section of the gonopodium at the top. The arrow points to a giant collagen fibre.

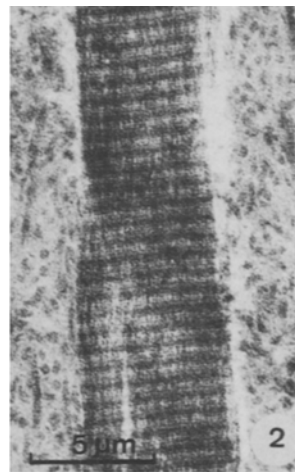


Fig. 2. High magnification of a giant collagen fibre, which gives an impression of the characteristic period and band pattern of the mature collagen.

were embedded in araldite and vestopal W, cut with an ultratome Om U 3 (Reichert) and later examined in an EM 9S (Zeiss) at 60 kV.

Many filaments and collagen fibres take part in the formation of gonopodium. Filaments occur not only in epidermal cells, but also in the central supporting tissue. At the top of the gonopodium filaments and collagen fibres increase in number and compactness.

In epidermal cells collagen fibres did not occur, but other filaments could be identified there. On the other hand, a thin course of collagen fibres lies below the basal lamina. The collagen fibres are arranged in 20–30 layers. 3–5 layers always cross one another at an angle of 90°. In this way they form a texture. At the top of the gonopodium there are collagen fibres, too, but they do not show any texture.

In the apical part of the gonopodium giant collagen fibres are a peculiarity. They are aligned in the transversal axis of the gonopodium (figure 1). Giant collagen fibres measure up to 150 µm in length and 6 µm in diameter (figures 1 + 2). In longitudinal section the fibres show the characteristic period and band pattern of mature collagen (figure 2). The giant collagen fibres are embedded in a great number of

minute collagen fibrils, which are arranged irregularly. Most of the minute collagen fibrils are synthesized from osteoblasts. These collagen fibrils correspond to the 'pre-osseous matrix' of light microscopy⁶. Collagen fibrils, giant collagen fibres and cytofilaments reinforce the gonopodium, which is stressed strongly during copulation. The place of synthesis of the giant collagen fibres is still unknown.

- 1 This research was supported by the Deutsche Forschungsgemeinschaft. Address for reprints: Forschungsgruppe Dermatologie der Universität Heidelberg, Im Neuenheimer Feld 324, D-6900 Heidelberg.
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Electrical resistance and spike activity in tarsal chemosensilla of *Phormia regina* (Meig.)¹

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Summary. The relationship between hair electrical resistance and responsiveness to stimulation has been investigated in the tarsal chemosensilla of *Phormia regina*. Results showed that the lower the hair electrical resistance, the higher is the spike-firing frequency.

The electrical resistance of the chemosensory hairs of the blowfly, *Phormia regina*, has been taken into account in previous research studies on the hypothesis that reliable information about the responsiveness of hairs to external stimulants may be gained by measuring it^{2–5}. This hypothesis has been based mainly on the following assumptions: 1. the viscous layer that separates the external environment from the chemosensory dendrites at the hair tip may be of a mucopolysaccharide nature; 2. by acting as a barrier, this layer could be the major factor in modulating chemical fluxes from the external environment to the chemosensory

dendrites, and consequently could be responsible for setting hair electrical resistance. The effectiveness of external chemicals as stimulants could thus be evaluated by measuring electrical resistance.

As regards assumption 1, preliminary experiments carried out in our laboratory but not yet published, have indeed proved that the apical viscous layer is of a mucopolysaccharide nature. As far as assumption 2 is concerned, we undertook the present investigation in order to make a direct study of the relationship between the electrical resistance and responsiveness of chemosensory hairs.

Firing frequency of the spike discharges recorded from tarsal chemosensory hairs of *Phormia*, following stimulation with NaCl solutions, was taken into account in evaluating hair responsiveness. 7- to 10-day-old adult blowflies, *Phormia regina*, were used. The insects were reared in our laboratory at 24 °C, 75% relative humidity, and fed with granulated sucrose and water. The chosen specimens were deprived of food 24 h before the experiments. Electrical resistance measurements were performed on D₅ tarsal hairs according to a method previously described by Stürckow⁶. The tip-recording procedure⁷ was adopted for recording hair spike activity. The stimulating-recording micropipette was filled with 1 M NaCl. Spike-firing frequency was measured in the tonic phase of discharge for a 1 sec period starting 200 msec after the onset of the stimulation. Almost all the spikes comprising the discharges were of the same size and shape, only a few spikes being of smaller amplitude. Only the larger spikes were taken into account. As shown in the table, some hairs failed to respond to stimulation with 1 M NaCl (inoperative hairs). Their electrical resistance was significantly higher than that of the hairs in which NaCl stimulation evoked spike-firing activity (operative hairs). A regression line has been calculated by relating electrical resistance to spike-firing frequency in the operative hairs. This regression line showed that the lower the hair electrical resistance, the higher the spike-firing frequency. In fact, the correlation coefficient was equal to 0.645 and significantly $\neq 0$ according to Student's *t* test ($p < 0.001$ on 86 experiments). Failure to respond to chemical stimulation by a given number of chemosensilla has already been observed in labellar and wing chemoreceptor hairs of *Phormia*^{8,9}. In agreement with the results of the present study on tarsal hairs, the electrical resistance of the inoperative wing hairs proved to be higher than that of the operative ones⁹. Here the mucopolysaccharide layer at the

hair tips may be the important modulating factor, as indicated by previous experiments in which the hair tips were cut off^{4,5}. It is consequently likely that, when hair electrical resistance exceeds a threshold level (or, in other words, when fluxes of stimulants from the external environment to the chemosensory dendrites are reduced below a given rate), the concentration of the stimulants at the chemoreceptive sites is too low to operate the chemoreceptor unit. Furthermore, our data on operative hairs demonstrate that, below threshold value, hair electrical resistance varies in a given range, and its variations influence hair responsiveness. It is very likely that, before reducing stimulant fluxes and consequently stimulant concentration below a critical non-operative level, an increase in hair electrical resistance can simply cause a reduction in spike-firing frequency, as may be deduced from the regression line of spike-firing frequency versus hair electrical resistance indicated previously.

In conclusion, our data directly demonstrate that a relationship between hair electrical resistance and responsiveness does exist in the chemosensilla of *Phormia*. Hair electrical resistance measurement is a very simple and quick procedure that can be widely adopted in chemosensitivity studies, chiefly when chemosensory hair responsiveness has to be tested in large populations of flies, or when responsiveness variations in a single chemosensillum are to be monitored for a given period of time. Electrical resistance measurements in fact require only very short periods of contact between hair tips and test solutions, thus reducing adaptation phenomena and allowing measurements to be repeated several times on the same hair without influencing its bioelectrical features.

Number of operative and inoperative tarsal D₅ hairs of *Phormia regina* Meig. and their electrical resistance when tested with 1 M NaCl (mean value \pm SE)

	Number of hairs	Electrical resistance (M Ω)
Operative hairs	86 (74.24%)	26.02 \pm 0.11
Inoperative hairs	32 (25.76%)	100.90 \pm 9.85

Electrical resistance of the operative hairs differs significantly from that of the inoperative ones (Student's '*t*' test, $p < 0.001$). Number of experiments = 118.

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Antifreeze agents in the body fluid of winter active insects and spiders¹

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Summary. Insects and spiders which are active at subzero temperatures on snow in winter are found to be protected against internal freezing by antifreeze agents present in their body fluid. The body fluid has a melting point of about -1 °C, but the antifreeze agents prevent growth of ice crystals at temperatures down to -6 to -7 °C.

One of the most peculiar features of insects and spiders which are active on snow in the winter is their ability to perform complex biological activity at low temperatures. The snow scorpionfly *Boreus westwoodi* and the spider *Bolyphantes index* perform activities such as mating, web

construction and feeding at temperatures below the melting point of their body fluid^{2,3}, and thus, they appear to be active in a supercooled state. Because of these extreme physical conditions and the permanent and intimate contact between the animals and external ice crystals, they